STUDY TITLE

1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP):
Primary Eye Irritation in Rabbits

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2400 (1998)

AUTHOR

Melissa Slonina, BS

STUDY COMPLETED ON

October 10, 2017

PERFORMING LABORATORY

Product Safety Labs

LABORATORY STUDY NUMBER

46321

SPONSOR

Halocarbon Products Corporation
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP)

This study meets the requirements of U.S. EPA GLP (TSCA): 40 CFR Part 792, 1989. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: M. Slonina Date: 10/10/17

Name of Signer: Melissa Slonina, BS

Name of Company: Product Safety Labs

Sponsor: _____ Date: _____

Name of Signer: _____

Name of Company: Halocarbon Products Corporation

Submitter: _____ Date: _____

Name of Signer: _____

Name of Company: Halocarbon Products Corporation

QUALITY ASSURANCE STATEMENT

The Product Safety Labs' Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Performed By	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	A. Adamiec; A. Villagran	Oct 1, 2013[1]; Sep 15, 2017	Oct 1, 2013; Sep 18, 2017
In-process inspection: *72-hour scoring and terminal body weights*	M. Zakrzewski	Sep 7, 2017	Sep 7, 2017
Raw data audit	A. Villagran	Sep 15, 2017	Sep 18, 2017
Draft report review	A. Villagran	Sep 15, 2017	Sep 18, 2017

Final report reviewed by:

Alicia Villagran

Alicia Villagran, RQAP-GLP
Quality Assurance Auditor
Product Safety Labs

Oct. 10, 2017
Date

[1] PSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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1,1,1,3,3,3-HEXAFLUORO-2-METHOXYPROPANE (HFMOP): PRIMARY EYE IRRITATION IN RABBITS

PROTOCOL NO.:	P324
STUDY NUMBER:	46321
SPONSOR:	Halocarbon Products Corporation 1100 Dittman Court North Augusta, SC 29861
TEST SUBSTANCE IDENTIFICATION:	1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP) Lot #: HFMOP17004
DATE RECEIVED:	August 18, 2017
PSL REFERENCE NO.:	170818-3R
STUDY INITIATION DATE:	August 21, 2017
DATES OF TEST:	September 4 - September 7, 2017
NOTEBOOK NO.:	46321: pages 1-31

1. PURPOSE

To provide information on the irritation likely to arise from a single instillation of 1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP) into the eye.

2. SUMMARY

A primary eye irritation test was conducted with rabbits to determine the potential for 1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP) to produce irritation from a single instillation via the ocular route. Under the conditions of this study, the test substance is classified as practically non-irritating to the eye.

One-tenth of a milliliter of the test substance was instilled into the right eye of three healthy rabbits. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the Draize method of scoring (Draize, Woodard, & Calvery, 1944; see Table 4).

One hour after test substance instillation, minimal conjunctivitis was noted for one treated eye, which cleared by 24 hours. There was no corneal opacity or iritis observed in any treated eye during this study.

The incidence of positive effects, severity and reversibility are detailed below:

Time Post Instillation	Incidence of Positive Effects		
	Corneal Opacity	Iritis	Conjunctivitis
1 hour	0/3	0/3	0/3
24 hours	0/3	0/3	0/3
48 hours	0/3	0/3	0/3
72 hours	0/3	0/3	0/3

Time Post Instillation	Severity of Irritation – Mean Score
1 hour	0.7
24 hours	0.0
48 hours	0.0
72 hours	0.0

3. MATERIALS

A. Test Substance

The test substance, identified as 1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP), Lot #: HFMOP17004, was received on August 18, 2017, and was further identified with PSL Reference Number 170818-3R. The test substance was stored at room temperature. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: 1,1,1,3,3,3-hexafluoro-2-methoxypropane - >99.99%, CAS #13171-18-1

Physical Description: Clear liquid

pH: neutral molecule

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not applicable

B. Animals

3.B.1 Number of Animals: 3

3.B.2 Sex: Female. All animals assigned to test were nulliparous and non-pregnant.

3.B.3 Species/Strain: Rabbit/New Zealand albino.

3.B.4 Age/Body Weight: Young adult (13 weeks)/2400-2741 grams at experimental start.

3.B.5 Source: Received from Robinson Services, Inc. on August 23, 2017.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging which conforms to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Enrichment (e.g., toy) was placed in each cage. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature and Relative Humidity Ranges: 20-23°C and 68-74%, respectively.

4.A.3 Animal Room Air Changes/Hour: 12. Airflow measurements are evaluated regularly and the records are kept on file at Product Safety Labs.

4.A.4 Photoperiod: 12-hour light/dark cycle

4.A.5 Acclimation Period: 12 days

4.A.6 Food: Envigo Teklad Global High Fiber Rabbit Diet® #2031. A designated amount of the diet (approximately 150 grams/day) and a Premium Timothy Cube™ (Ontario Dehy Inc.) were available to each rabbit.

4.A.7 Water: Filtered tap water was supplied *ad libitum*.

4.A.8 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Product Safety Labs.

B. Identification

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

4.B.2 Animal: A number was allocated to each rabbit on receipt and a stainless steel ear tag bearing this number was attached to the animal. This number, together with a sequential animal number assigned to study 46321, constituted unique identification. Only the sequential animal number is presented in this report.

5. PROCEDURE

A. Preparation and Selection of Animals

Prior to test initiation, both eyes of a group of animals were examined using a white light source and a fluorescein dye procedure. One drop of ophthalmic fluorescein sodium dye was instilled into both eyes of each rabbit. The eyes were rinsed with physiological saline (0.9% NaCl) after instillation of the fluorescein and then evaluated for corneal damage using an ultraviolet light source. Prior to test substance instillation, the eyes were re-examined and scored for abnormalities according to the "Scale for Scoring Ocular Lesions" (Draize et al., 1944; see Table 4). Three healthy, naive animals (not previously tested) without pre-existing ocular irritation were selected for test.

A systemic analgesic (Buprenorphine SR®) was administered to relieve potential discomfort associated with eye irritation which provides therapeutic relief for periods of up to 76 hours.

Prior to test substance instillation, 0.1 mg/kg of body weight of the analgesic was administered to the animals and at appropriate intervals to maintain therapeutic blood levels.

B. Preparation of Test Substance

The test substance was instilled as received and mixed well prior to use.

The pH was determined for the test substance prior to the instillation and was within a pH range of 2 and 11.5, therefore testing proceeded. The procedure used and the results are retained in the raw data.

C. Instillation

Prior to instillation, 1-2 drops of ocular anesthetic (Tetracaine Hydrochloride Ophthalmic Solution USP, 0.5%) were placed into both the treated and control eye of each animal. One-tenth of a milliliter of the test substance was then instilled into the conjunctival sac of the right eye of each rabbit by pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for about one second before releasing to minimize loss of the test substance. The other eye of each rabbit remained untreated with the test substance and served as a control. The rabbits were then returned to their designated cages.

D. Ocular Scoring

Ocular irritation was evaluated using a white light source in accordance with the Draize method of scoring (Draize et al., 1944; see Table 4) at 1, 24, 48, and 72 hours post-instillation. The fluorescein dye evaluation procedure described in Section 5.A. was used in the treated eye at 24 hours to verify the absence of corneal damage. Individual scores were recorded for each animal. In addition to observations of the cornea, iris and conjunctivae, any other observed lesions were noted. The average score for all rabbits at each scoring period was calculated to aid in data interpretation.

E. Classification of Eye Scores

The time interval with the highest mean score (Maximum Mean Total Score - MMTS) for all rabbits was used to classify the test substance by the system of Kay and Calandra (Kay & Calandra, 1962).

F. Cage-Side Observations

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

G. Body Weights

Individual weights of animals were recorded shortly before instillation of the test substance (initial) and at the completion of testing (terminal).

H. Study Termination

Once testing was complete, the animals were released for euthanasia and humanely euthanized.

6. STATISTICAL ANALYSIS

Statistical analysis was limited to the calculation of the mean irritation scores.

7. STUDY CONDUCT

This study was conducted at Product Safety Labs' (PSL) test facility at 2394 US Highway 130, Dayton, New Jersey 08810. The Study Director for this study was Melissa Slonina, BS. The primary scientist for this study was Monika Abraham, BA. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- U.S. EPA GLP: Toxic Substances Control Act (TSCA): 40 CFR Part 792, 1989

and based on the following testing guideline:

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.2400 (1998)

8. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Labs Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

9. AMENDMENTS TO THE PROTOCOL

None.

10. DEVIATIONS FROM THE PROTOCOL

None.

11. FINAL REPORT AND RECORDS TO BE MAINTAINED

Information on care of the test system, equipment maintenance and calibration, storage, usage, and disposition of the test substance, and all other records that would demonstrate adherence to the protocol will be maintained. Facility records which are not specific to the subject study will be maintained by the testing facility and archived according to PSL SOP.

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at PSL, is maintained in the PSL Archives. PSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by PSL.

12. RESULTS

Individual body weights are presented in Table 1. Individual cage-side observations are presented in Table 2. Individual ocular irritation scores are presented in Table 3. The Draize Scale for Scoring Ocular Lesions is presented in Table 4. The Kay and Calandra scheme for classifying eye irritants is presented in Table 5.

All animals appeared active and healthy during the study. Although one animal lost body weight, the two remaining animals gained body weight during the study. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse clinical effects, or abnormal behavior.

One hour after test substance instillation, minimal conjunctivitis was noted for one treated eye, which cleared by 24 hours. There was no corneal opacity or iritis observed in any treated eye during this study.

The Maximum Mean Total Score of 1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP) is 0.7.

13. CONCLUSION

Under the conditions of this study, 1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP) is classified as practically non-irritating to the eye.

14. REFERENCES

Draize, J.H., Woodard, G., & Calvery, H.O. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.*, 82, 377-390.

Kay, J.H., & Calandra, J.C. (1962). Interpretation of eye irritation tests. *J. Soc. Cos. Chem.*, *13*, 281-289.

National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals (8th ed.)*. Washington, DC: The National Academies Press.

SIGNATURE

1,1,1,3,3,3-Hexafluoro-2-methoxypropane (HFMOP)

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

M. Slonina
Melissa Slonina, BS
Study Director
Product Safety Labs

10/10/17
Date

TABLE 1: INDIVIDUAL BODY WEIGHTS

Animal No.	Sex	Body Weight (g)	
		Initial	Terminal
3401	F	2741	2666
3402	F	2656	2730
3403	F	2400	2510

TABLE 2: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Animal Sex	Observation	Day of Observation (x=observation is present)			
			0	1	2	3
3401	F	Active and healthy	x	x	x	x
3402	F	Active and healthy	x	x	x	x
3403	F	Active and healthy	x	x	x	x

TABLE 3: INDIVIDUAL SCORES FOR OCULAR IRRITATION

	Rabbit No.: 3401 (Female)				Rabbit No.: 3402 (Female)				Rabbit No.: 3403 (Female)			
	Hour				Hour				Hour			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Cornea												
A. Opacity	0	0[1]	0	0	0	0[1]	0	0	0	0[1]	0	0
B. Area	4	4	4	4	4	4	4	4	4	4	4	4
(AxB)x5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
Ax5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	0	0	0	0	0	0	0	0	0	0	0	0
B. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C. Discharge	1	0	0	0	0	0	0	0	0	0	0	0
(A+B+C)x2	2	0	0	0	0	0	0	0	0	0	0	0
Total	2	0	0	0	0	0	0	0	0	0	0	0

[1] Ophthalmic fluorescein sodium dye was used to verify the absence of corneal opacity.

TABLE 4: SCALE FOR SCORING OCULAR LESIONS[1]

1. Cornea

A. Opacity-degree of density (area most dense taken for reading)

No Opacity 0
Scattered or diffuse area, details of iris clearly visible ..... 1[2]
Easily discernible translucent areas, details of iris slightly obscured ..... 2[2]
Opalescent areas, no details of iris visible, size of pupil barely discernible ..... 3[2]
Opaque, iris invisible ..... 4[2]

B. Area of cornea involved

One quarter (or less) but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

A X B X 5 Total Maximum = 80

2. Iris

A. Values

Normal 0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive) ..... 1[2]
No reaction to light, hemorrhage, gross destruction (any or all of these) ..... 2[2]

A X 5 Total Maximum = 10

3. Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal 0
Vessels definitely injected above normal 1
More diffuse, deeper crimson red, individual vessels not easily discernible ..... 2[2]
Diffuse beefy red ..... 3[2]

B. Chemosis

No swelling 0
Any swelling above normal (includes nictitating membrane) 1
Obvious swelling with partial eversion of lids ..... 2[2]
Swelling with lids about half-closed ..... 3[2]
Swelling with lids about half-closed to completely closed ..... 4[2]

C. Discharge

No discharge 0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs, and considerable area around the eye 3

Score (A + B + C) X 2 Total Maximum = 20

Total Maximum Score: 110 represents the sum of all scores obtained for the cornea, iris and conjunctivae.

[1] Draize et al., 1944

[2] These scores represent a positive response.

TABLE 5: CLASSIFICATION OF EYE IRRITATION SCORES

MMTS	Irritation Classification	Requirement For Maintenance of Classification[1]
0.0 - 0.5	non	Up to 0.5 at 1 hour with zeros at 24 hours; otherwise, increase one level
0.6 - 2.5	practically non	with zeros at 24 hours; otherwise, increase one level
2.6 - 15.0	minimally	with zeros at 48 hours; otherwise, increase one level
15.1 - 25.0	mildly	with zeros at 96 hours; otherwise, increase one level
25.1 - 50.0	moderately	with 7 day mean $\leq$ 20 and individual total scores $\leq$ 10 in at least 60% of the rabbits with no total score >30; otherwise, increase one level
50.1 - 80.0	severely	with 7 day mean $\leq$ 40 and individual total scores $\leq$ 30 in at least 60% of the rabbits with no total score > 60; otherwise, increase one level
80.1 - 100.0	extremely	with 7 day mean $\leq$ 80 and individual total scores $\leq$ 60 in at least 60% of the rabbits with no total score >100; otherwise, increase one level
100.1 - 110	maximally	with 7 day mean > 80 and individual total scores > 60 in at least 60% of the rabbits; otherwise, decrease one level

[1] Kay & Calandra, 1962